



- 1 -

19. Japanese Patent Office

12. Publication of an unexamined patent application (A)
11. Publication number of the unexamined patent application:
10-117787

43. Date of publication: May 12, 1998
51. Int. Cl.⁶: C12N 15/09, A61K 38/46, 38/48, C07K 14/745,
C12N 5/10, C12N 9/64
//A61K 38/43, (C12N 9/64, C12R 1:91)

Classification symbols: ZNA, ACA

FI Classification: C12N 15/00, C07K 14/745, C12N 9/64,
A61K 37/54, 37/553, C12N 5/00, A61K 37/465

Application for examination: filed

Number of inventions: 1 (total 47 pages)

21. Application number: 9-247281
62. Divisional application data:
Divisional application from patent application No. 6-276832
22. Date of application: 16. 04. 1986
31. Priority number: 724311
32. Priority date: 17. 04. 1985
33. Priority state: U.S.
31. Priority number: 810002
32. Priority date: 16. 12. 1985
33. Priority state: U.S.

71. Applicant: 594185754
ZymoGenetics, Inc., 2121 North 35th Street, Seattle,
Washington 98103, U.S.A.
72. Inventor: Frederick S. Hagen, 3835 44th N.E., Seattle,
Washington 98105, U.S.A.
72. Inventor: Mark J. Murray, 2211 11th Avenue East, Seattle,
Washington 98102, U.S.A.
72. Inventor: Sharon J. Busby, 4109 Meridian N., Seattle,
Washington 98103, U.S.A.
72. Inventor: Kathleen L. Berkner, 3032 22nd Avenue W., Seattle,
Washington 98199, U.S.A.
72. Inventor: Margaret Y. Insley, 16860 N.E. 150th Street,
Woodinville, Washington 98072, U.S.A.
72. Inventor: Richard G. Woodbury, 15464 10th Avenue, N.E.,

Seattle, Washington 98155, U.S.A.

72. Inventor: Charles L. Gray, 8014 41st Avenue, N.E., Seattle,
Washington 98115, U.S.A.

74. Representative: Patent attorney Aoyama (and 2 others)

54. Title of the invention:

Method of producing protein having factor VII activity

57. Abstract:

Problem: Novel method of producing protein having factor VIIa activity.

Solution: Method characterized in that mammalian cells are cultured into which DNA coding for protein having factor VIIa activity after activation has been inserted, and protein is obtained from this culture fluid and activated.

PATENT CLAIMS:

1. A method of producing protein having biological activity for blood coagulation mediated by factor VIIa comprising growing in an appropriate culture medium mammalian host cells containing a DNA construct containing a nucleotide sequence coding for a protein having the same or essentially the same biological activity for blood coagulation as factor VIIa having the following amino acid sequence:

[see extra sheet]

isolating the protein product encoded by said DNA construct and produced by said mammalian host cells, and activating said protein product and generating protein which has the same or substantially the same biological activity for blood coagulation as factor VIIa.

2. A method according to claim 1, including amplification of said DNA construct by cotransfection of said host cells with a gene coding for dihydrofolate reductase, wherein said appropriate medium contains methotrexate.

3. A method according to claim 1, wherein said protein product is activated by reacting it with a proteolytic enzyme selected from the group consisting of factor XIIa, factor IXa, kallikrein, factor Xa, and thrombin.

| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1 | 5 | 10 | 15 | | | | | | | | | | | |
| Glu | Cys | Lys | Glu | Glu | Gln | Cys | Ser | Phe | Glu | Glu | Ala | Arg | Glu | Ile |
| 20 | 25 | 30 | | | | | | | | | | | | |
| Phe | Lys | Asp | Ala | Glu | Arg | Thr | Lys | Leu | Phe | Trp | Ile | Ser | Tyr | Ser |
| 35 | 40 | 45 | | | | | | | | | | | | |
| Asp | Gly | Asp | Gln | Cys | Ala | Ser | Ser | Pro | Cys | Gln | Asn | Gly | Gly | Ser |
| 50 | 55 | 60 | | | | | | | | | | | | |
| Cys | Lys | Asp | Gln | Leu | Gin | Ser | Tyr | Ile | Cys | Phe | Cys | Leu | Pro | Ala |
| 65 | 70 | 75 | | | | | | | | | | | | |
| Phe | Glu | Gly | Arg | Asn | Cys | Glu | Thr | His | Lys | Asp | Asp | Gln | Leu | Ile |
| 80 | 85 | 90 | | | | | | | | | | | | |
| Cys | Val | Asn | Glu | Asn | Gly | Gly | Cys | Glu | Gln | Tyr | Cys | Ser | Asp | His |
| 95 | 100 | 105 | | | | | | | | | | | | |
| Thr | Gly | Thr | Lys | Arg | Ser | Cys | Arg | Cys | His | Glu | Gly | Tyr | Ser | Leu |
| 110 | 115 | 120 | | | | | | | | | | | | |
| Leu | Ala | Asp | Gly | Val | Ser | Cys | Thr | Pro | Thr | Val | Glu | Tyr | Pro | Cys |
| 125 | 130 | 135 | | | | | | | | | | | | |
| Gly | Lys | Ile | Pro | Ile | Leu | Glu | Lys | Arg | Asn | Ala | Ser | Lys | Pro | Gln |
| 140 | 145 | 150 | | | | | | | | | | | | |
| Gly | Arg | Ile | Val | Gly | Gly | Lys | Val | Cys | Pro | Lys | Gly | Glu | Cys | Pro |
| 155 | 160 | 165 | | | | | | | | | | | | |
| Trp | Gln | Val | Leu | Leu | Leu | Val | Asn | Gly | Ala | Gln | Leu | Cys | Gly | Gly |
| 170 | 175 | 180 | | | | | | | | | | | | |
| Thr | Leu | Ile | Asn | Thr | Ile | Trp | Val | Val | Ser | Ala | Ala | His | Cys | Phe |
| 185 | 190 | 195 | | | | | | | | | | | | |
| Asp | Lys | Ile | Lys | Asn | Trp | Arg | Asn | Leu | Ile | Ala | Val | Leu | Gly | Glu |
| 200 | 205 | 210 | | | | | | | | | | | | |
| His | Asp | Leu | Ser | Glu | His | Asp | Gly | Asp | Glu | Gln | Ser | Arg | Arg | Val |
| 215 | 220 | 225 | | | | | | | | | | | | |
| Ala | Gln | Val | Ile | Ile | Pro | Ser | Thr | Tyr | Val | Pro | Gly | Thr | Thr | Asn |
| 230 | 235 | 240 | | | | | | | | | | | | |
| His | Asp | Ile | Ala | Leu | Leu | Arg | Leu | His | Gln | Pro | Val | Val | Leu | Thr |
| 245 | 250 | 255 | | | | | | | | | | | | |
| Asp | His | Val | Val | Pro | Leu | Cys | Leu | Pro | Glu | Arg | Thr | Phe | Ser | Glu |
| 260 | 265 | 270 | | | | | | | | | | | | |
| Arg | Thr | Leu | Ala | Phe | Val | Arg | Phe | Ser | Leu | Val | Ser | Gly | Trp | Gly |
| 275 | 280 | 285 | | | | | | | | | | | | |
| Gln | Leu | Leu | Asp | Arg | Gly | Ala | Thr | Ala | Leu | Glu | Leu | Met | Val | Leu |
| 290 | 295 | 300 | | | | | | | | | | | | |
| Asn | Val | Pro | Arg | Leu | Met | Thr | Gln | Asp | Cys | Leu | Gln | Gln | Ser | Arg |
| 305 | 310 | 315 | | | | | | | | | | | | |
| Lys | Val | Gly | Asp | Ser | Pro | Asn | Ile | Thr | Glu | Tyr | Met | Phe | Cys | Ala |
| 320 | 325 | 330 | | | | | | | | | | | | |
| Gly | Tyr | Ser | Asp | Gly | Ser | Lys | Asp | Ser | Cys | Lys | Gly | Asp | Ser | Gly |
| 335 | 340 | 345 | | | | | | | | | | | | |
| Gly | Pro | His | Ala | Thr | His | Tyr | Arg | Gly | Thr | Trp | Tyr | Leu | Thr | Gly |
| 350 | 355 | 360 | | | | | | | | | | | | |
| Ile | Val | Ser | Trp | Gly | Gln | Gly | Cys | Ala | Thr | Val | Gly | His | Phe | Gly |
| 365 | 370 | 375 | | | | | | | | | | | | |
| Val | Tyr | Thr | Arg | Val | Ser | Gln | Tyr | Ile | Glu | Trp | Leu | Gln | Lys | Leu |
| 380 | 385 | 390 | | | | | | | | | | | | |
| Met | Arg | Ser | Glu | Pro | Arg | Pro | Gly | Val | Leu | Leu | Arg | Ala | Pro | Phe |
| 395 | 400 | 405 | | | | | | | | | | | | |

Pro

Figure 5 illustrates the Factor VII cDNA sequence of λ VII 2463.
Figure 6 illustrates the Factor VII cDNA sequence of λ VII 2463.
Figure 7 illustrates the Factor VII cDNA sequence of λ VII 2463.